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VVD-041 - SUBTYPING OF SWINE INFLUENZA VIRUSES USING A HIGH-THROUGHPUT REAL TIME PCR PLATFORM

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Introduction

Swine influenza is a respiratory disease caused by multiple subtypes of influenza A virus (IAV). The genome of IAV consists of 8 segments and subtype classification is based on the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). In Denmark, the influenza screening test and subsequent subtyping is performed by real time RT-PCR (RT-qPCR) but several assays are needed to cover the wide range of circulating subtypes which is expensive, resource and time demanding. To mitigate these restrictions the high-throughput qPCR platform BioMark (Fluidigm) has been explored. The BioMark platform uses less sample and reagent volume compared to standard qPCR platforms and allows for up to 9,216 parallel reactions on one chip.

Materials and Methods

A total of 14 PCR assays specific for the different subtypes of HA and NA genes relevant for swine influenza and 6 assays specific for the internal genes of IAV were validated and optimised to run under identical reaction conditions and assembled on a dynamic array chip (Fluidigm).

Results

The sensitivity and specificity of the chip was assessed by testing cell culture isolates and field samples with known subtypes (based on sequencing). The results revealed that the performance of the dynamic chip was similar to conventional real time analysis.

Discussion and Conclusion

Application of the chip for subtyping of swine influenza has resulted in a significant reduction in time, cost and working hours. Thereby, it is possible to offer diagnostic services with reduced price and turnover time which will facilitate choice of vaccines and by that lead to reduction of antibiotic used.